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**(54) Regulated genes by stimulation of chondrocytes with 1L-1beta**

(57) The present invention refers to the novel use of osteopontin, calnexin and TSG-6 gene product in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1 $\beta$  and their use in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues.

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## Description

The present invention refers to the novel use of osteopontin and cannexin in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1 $\beta$  and their use in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues.

Among the diverse biological effect of interleukin-1 (IL-1), are its actions on the metabolism of many connective tissue cell types including articular chondrocytes. IL-1 inhibits proteoglycan (PG) synthesis by chondrocytes and stimulates production of prostaglandin E<sub>2</sub> and metallo-proteinases capable of degrading matrix macromolecules. From experimental results, and from analysis of IL-1, PG fragments and proteolytic enzymes in inflamed joints, it was deduced that IL-1 plays a role in cartilage degradation in osteoarthritis and rheumatoid arthritis (Benton HP & Tyler JA, 1988, *Biochem. Biophys. Res. Comm.* 154, 421-428; Aydelotte MB et al., *Conn. Tiss. Res.* 28, 143-159; Wood DD et al., *Arthritis Rheum.* 26, 975-983; Lohmander LS et al., *Trans Orthop. Res. Soc.* 17, 273). Matrix metalloproteinases are potential candidates for drug interaction at the enzyme level, but relevant molecular targets interfering with earlier processes leading to cartilage degradation are still lacking. Therefore, one objective of the present invention was to identify potential targets for drug modification of IL-1 $\beta$  induced cartilage degradation on the RNA level of human articular chondrocytes from osteoarthritic cartilage.

As an initial attempt to investigate differentially expressed genes in diseased cartilage, total RNA from IL-1 $\beta$  stimulated and unstimulated human chondrocytes was subjected to differential display of mRNA by reverse transcription and polymerase chain reaction (DDRT-PCR). This method can be used to identify and isolate those genes that are differentially expressed in two cell populations (Liang P & Pardee AB 1992, *Science* 257, 967-971; Liang P et al., AB 1993, *Nucl. Acids Res.* 21, 3269-3275; Bauer D et al. 1993, *Nucl. Acids Res.* 21, 4272-4280). The key element is to use a set of oligonucleotide primers, one hybridizing to the polyadenylated tail of mRNAs, the other being arbitrary decamers that anneal at different positions relative to the first primer. mRNA subpopulations defined by these primer pairs are amplified after reverse transcription and resolved on DNA sequencing gels. Band patterns are created, which are characteristic for each RNA population extracted from the cell population under study. For example, 100 different primer combinations should generate a total of approximately 10,000 PCR products for each population, which should represent about the half of all expressed cellular genes. A comparison of the band pattern obtained from two cell populations reveals differentially displayed bands which correspond to differentially expressed genes. Subsequently, differentially displayed bands can be extracted from the gel, reamplified, subcloned and sequenced.

Due to its extreme sensitivity, the appearance of artifactual bands is an inherent problem of the DDRT-PCR method used according to the present application. An additional problem is also the evaluation of complex gene expression patterns. Yet another problem of the present invention is that only minute amounts of RNA are available.

Therefore, it was particularly surprising that the DNA TAU1/1 with the sequences

TAU1/1(1)	
ACATCACCTC ACACATGGAA ACCGAGGAGT TGAATGGTGC ATACAAGGCC ATCCCCGTTT	60
CCCAAGGACCT GAAACCGCCT TCTGATTGGG ACAGCCGTGG GAAAGGACAGT TATGAAACGA	120
GTCAGCTGGA TGACCCAGCT GCTGAAACCC ACAGGCCACAA GCAGTCCAGA TTATATAAGC	180
GGAAA	185

and	
TAU1/1(2)	
CTAAATGCCAA AGTGAGAAAT TGTATTTTTT CTCCCTTTAA TTGACCTCAG AAGATGCCACT	60
ATCTAATTCA TGAGAAATAC GAAATTTCAG GTCTTTATCT TCTTCCTTAC TTTGGGG	118

and the DNA TTU2/2 with the sequence

5	AACCACTATT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT TGTTCCTTA	60
	TCAGTAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG CATGTTTTC TTCACTAGATA	120
	CCCGTTTTGG GGTTTTGGG TCGGAAGATG AATGGCATT ATAGTCCTCT CCACATTAT	180
	CTG	

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are 100 % identical to human osteopontin cDNA and 97.2 identical to human calnexin, respectively. This demonstrates that the experimental approach of the present invention worked efficiently, i.e. the use of 100 different primer combinations (25 oligodecamer primers, 4T<sub>1</sub>MN-primers) generated a total of approximately 10,000 PCR products for each population which represent 53 % of all expressed cellular genes. 123 PCR bands out of 10,000 appeared as differentially expressed bands. 53 of the original 123 PCR bands were reproducibly displayed by comparing the PCR band patterns from two patients; of those 68 % arose from IL-1 $\beta$  stimulated chondrocytes.

It was further found that osteopontin is a secreted highly acidic phosphoprotein of 32 kd (Denhardt and Guo (1993) FASEB J, 7, 1475-1482) is surprisingly downregulated in IL-1 $\beta$  stimulated human chondrocytes. This means that osteopontin is involved in IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis.

20 Osteoarthritis is characterized as a slowly progressing matrix degeneration with continuing degradation of collagens and proteoglycans and subsequent release of matrix fragments into the synovial fluid. Any disturbance of the normal chondrocyte matrix interactions, for example through a loss of osteopontin, could cause an altered signaling through the integrin alpha<sub>1</sub>beta<sub>1</sub> and thus changed cellular responses leading to early steps of matrix degradation.

Therefore, one embodiment of the present invention is the use of osteopontin itself or parts thereof, antibodies against it or nucleic acids such as DNA or RNA or parts thereof coding for osteopontin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis. According to the present application the term "parts" means either at least 8, preferably 12, in particular 15 amino acids in case of proteins or 6-100, preferably 10-40, in particular 12-25 nucleic acids in case of DNA or RNA as hybridization probes. The methods of diagnosing such diseases will be described infra. In addition, quantification on the protein level is possible with osteopontin specific antibodies on Western blots, in immunochemistry, FACS analysis or ELISA based assay systems. The present invention refers also to a diagnosis aid or a pharmaceutical for such use. Osteopontin can be produced for example recombinantly through expression in prokaryotes, in insect cells in mammalian cells or in mammalian cells using Vaccinia as detailed in Ausubel et al. 1994 [Current protocols in molecular biology, Chapter 16, John Wiley & Sons, Inc]. The cDNA of Osteopontin is e.g. disclosed in Young et al. (1990), *Genomics* 7, 491 - 502.

35 Antibodies against osteopontin can be generally produced for example by the method of Neil GA & Urnovitz HB (Trends in Biotechnology, 6, 209-213, 1988) or Köhler G & Milstein C (Nature, 256, 52-53, 1975).

Also calnexin which is an integral membrane protein of 88 kd (Bergeron et al. (1994) TIBS 19, 124-128) is surprisingly downregulated in IL-1 $\beta$  stimulated human chondrocytes compared to unstimulated chondrocytes. This means also that calnexin is involved in IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis. In addition, a downregulation of the calnexin synthesis would cause a reduced amount of correctly and completely folded proteoglycans because calnexin is a new type of molecular chaperone that associates with incompletely folded proteins such as proteoglycans. Proteoglycans are highly glycosylated glycoproteins which are of central importance for the maintenance of the cartilage tissue integrity.

Hence, an additional embodiment of the present invention is the use of calnexin itself, or parts thereof antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

Calnexin can be produced for example recombinantly as described above for osteopontin. The cDNA of Calnexin is e.g. disclosed in Galvin et al. (1992), Proc. Natl. Acad. Sci. USA 89, 8452 - 8456. The production of said antibodies are also generally described above.

#### Potential role of identified cDNA fragments in IL-1 mediated cellular processes TSG-6

55 A homology search in the GenBank and EMBL databases revealed a 99.5 % sequence identity of fragment TAU7/2(c) with the gene coding for human TSG-6. TSG-6 (TNF stimulated gene 6) was originally isolated by differential cDNA library screening as a TNF induced gene sequence from human fibroblasts (Lee et al., 1990). It was further characterized by Lee et al (1992) as a TNF and IL-1 inducible, secretory, 39 kDa glycoprotein with extensive sequence homology with a region implicated in hyaluronate binding, present in cartilage link protein, proteoglycan core proteins,

and the adhesion receptor CD44. With the ability to bind HA and with the most extensive sequence homology to CD44, TSG-6 belongs to the hyaladherin family. Wisniewski et al. (1993) detected high levels of TSG-6 protein in synovial fluids of patients with various forms of arthritis. Six normal control patients did not contain detectable TSG-6 protein in their joint fluid, whereas joint fluids from nine rheumatoid arthritis patients contained high, moderate or low levels of TSG-6.

5 Two patients with osteoarthritis had high levels of TSG-6 in their joint fluids. The apparent local source of TSG-6 in the joints are synoviocytes and chondrocytes (Wisniewski et al., 1993). Lee et al. (1992) speculated that TSG-6 could act as a competitive inhibitor of the interaction between CD44 and its ligand(s) and thus might influence the structural organization of the extracellular matrix of connective tissue, resulting in a destabilization of the proteoglycan aggregates.

Hence, an additional embodiment of the present invention is the use of TSG-6 gene product itself, or parts thereof 10 antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

#### 15 Fibronectin

A homology search in the GenBank and EMBL databases revealed a 100 % sequence identity of fragment TTQ201/c with the gene coding for human fibronectin.

20 Fibronectin is a 450 kD glycoprotein with various functions. It acts as an adhesive ligand, as growth or differentiation factor and has chemotactic properties. It is found in the extracellular matrix of most types of cells (Hynes R 1993. Fibronectins, In: Guidebook to the extracellular matrix and adhesion proteins. Editors: Kreis T, Vale R. Oxford University Press. 56-58). An enhanced accumulation of fibronectin and fragments derived from it are found in the synovial fluid and on the inflamed synovial and pannus surfaces in the knee joint of patients with rheumatoid arthritis (Dutu A, Vlaicu Rus V, Boloisici HD, Parasca I, Cristea A. 1986. Fibronectin in plasma and synovial fluid of patients with rheumatic diseases. Med. Interne 24, 61-68). Patients with osteoarthritis, as well, have greatly increased levels of fibronectin in their synovial fluid and on cartilage surfaces (Xie D-L, Meyers R, Homandberg GA. 1992. Fibronectin fragments in osteoarthritis synovial fluid. J. Rheumatology 19, 1448-1452). The intraarticular injection of fibronectin fragments causes a severe depletion of cartilage proteoglycans in vivo (Homandberg GA, Meyers R, Williams JM. 1993. Intraarticular injection of fibronectin fragments causes severe depletion of cartilage proteoglycans in vivo. J. of Rheumatology 20, 1378-1382), which is explained by the induced release of several proteinases, including stromelysin (Xie D-L, Hui F, Meyers R, Homandberg GA. 1994. Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: Stromelysin plays a major role in chondrolysis. Arch. Biochem. and Biophysics 311, 205-212). At high concentrations, fibronectin fragments enhance cartilage catabolism through release of cytokines, including IL-1 (Homandberg et al., personal communication).

30 In respect to these published data, the upregulation of fibronectin by IL-1 can be regarded as a positive feedback regulation, enhancing the self destructive potential of chondrocytes and synoviocytes. With this, fibronectin expression is a direct pharmacological target.

In addition, the sequencing of differentially displayed PCR products discovered also unknown DNA fragments which 35 correspond to differentially expressed genes with or without stimulation of chondrocytes with IL-1 $\beta$ .

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Therefore, another embodiment of the present invention is a DNA containing a DNA selected from the group consisting of

## 5 TA08/2 (2)

1	CCAAAGTTTT	CCAGGCAACCC	CAAGGGATA	CAGGGAGATC	AATGCACCA
51	AAATGGGAAA	AGRAAAAATAC	TTCGATGCAA	TGAACCAAAG	CCTTTTCGG
101	TTCAGTTCC	ATAATTCACT	GGTCAGTTT	AAGGCTGCCA	CTTGGG

## 10 TA016/1(2)

1	GACACGAACA	CCACATATT	TTATTGGAGG	CCCCATGGCT	CCTTGGAAAGC
51	CATTTGGAA	CCAAGGGGAC	CCACCTTTT		

## 15 TA016/2(2)

1	CTAAATATAT	TCTCTAACAA	GTAAATCTCT	TTCAAATCTA	TAGATAAAAAC
51	TAAAAGGATA	AGGAAACCGAG	GTAAACCGGA	CCTAGCCAT	TATGGCAATC
101	ATACTTGCTT	TTTAG			

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## TA017(C)

5           1   CATGAAATAT  TTCTTGAGGT  AATAAGCTTT  TACCAAGCTT  ATATTTTGG  
  51   GCAATTCACT  TACAATGAGA  AAAAAACACA  CCAAAAGACC  AAAAATTATA  
  
  101   AAAACTCACT  TTCTTGCAA  TCATAGACAT  TTGCATTATT  ATAGAACATT  
  151   CAAAACAAGTT  AGGTGGATAA  TTATGTCATA  TAGATAATAA  CGATGCAATT  
  201   TTTTAAATGT  ATGACCGATA  CTCCGTATAT  ACTTAGATAA  CTTATCCAGA  
  301   AACCTCACT  GTATTGAAAC  TTGCTGAGAG  AAATCAACAA  TAATTTAAC  
  351   AGATATGATC  ACAGNAAAAA  TTGATTCAT  ATCTTTTGC  ACTAAAACCT  
  401   TTATATTTAT  TT

15

## TA019(C)

20           1   AGAGCAGGGG  TATTCNCGG  TTCATACCGC  CATGGCTTAA  GAAGCAAAAG  
  51   TCATATACCT  TAGTAGTGGC  AAAGATNGAG  GAGATAAAA  AGAGCCTACC  
  101   CAAGCTGTG  TTGAAAGAAC  GGTCTTAGAT  AAAGAGGAC  CCTTCCAGAA  
  151   GNACAGAGAC  AGGCTAAGGG  TGATGCTGAG  GAARATGGCTC  AGAAGAAAACA  
  201   AGAGATTTAA

## TAU 1/1(2)

25           1   CTAAATGCAA  AGTGAGAAAT  TGTATTTTT  CTCCTTTAA  TTGACCTCAG  
  51   AAGATGCACT  ATCTAATTCA  TGAGAAATAC  GAAATTTCAG  GTGTTATCT  
  101   TCTTCCTTAC  TTTTGGGG

## TAU 1/1(1)

30           1   ACATCACCTC  ACACATGGAA  AGCGAGGAGT  TGAATGGTGC  ATACAAGGCC  
  51   ATCCCCCTT  CCCAAGGACTT  GAACCCGCT  TCTGATTGGG  ACAGCCGTGG  
  101   GAAGGGACAGT  TATGAAACGA  GTCAGCTGGA  TGACCAGAGT  GCTGAAACCC  
  151   ACAGCCCCCAA  CGAGTCAGAA  TTATATAAGC  GGAAA

35

## TAU1/2(2)

40           1   CCGGAAATGGG  GAGCAGAACTA  TAAGAACCGG  GACCAGTTTC  CTCTCTTTGT  
  51   GCCCTAGTC  CCCCCTCTTT  GTATACACCC  TCCATCCTGA  ATAGACTCTG  
  101   GTTCTCAGCG  TAACACCGAC  AACATTCAT  CCTGTTAGAGA  AACAAATGTT  
  151   AGCTCAGAAG  GACACAGCCT  TTGAATCATC  AGAGAGTT

## TAU 7/1(2)

45           1   GTTAAGAATA  ACTAAATAAA  AGTTTTAATT  AATTAGGAA  TATAAAAAAC  
  51   TATTACATT  TAATTTTATA  ACTGTATCTG  CCAAGCAACT  TTAAATATAA  
  101   TTTATTTAC

## TAU 7/1(1)

50           1   CACGCAATGT  GAAATAGGCA  CATAGGAAGA  ATGGGGAAAC  CATCCCCCTCA  
  51   AGCAATTATC  CTTGTGAGTTA  CAAGCAATCC  AATTACACTC  TTTGTATAT  
  101   TTTTAAATGT  ACAGTTAGGT  TATTA

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## TAU 7/2(C)

5           1   CCTTGAAGAT   GACCCAGGTT   NCTTGGCTGA   TTATGTTGAA   ATATAGACA  
       51   GTTACGATGA   TGTCCATGGC   TTTGTGGAA   GATACTGTGG   AGATGAGCTT  
      101   CCAGATGACA   TCATCAGTAC   AGGAATATTC   ATGACCTGA   AGTTTCTAAG  
      151   TGATGCTTCA   GTGACAGCTG   GAGGTTCCA   AATCAATAT   GTTGCAATGG  
      201   AT

## 10           TAU10(1)

15           1   GGAGATGACA   TTTGCTTTGG   GCAGAGGCAG   CTAGCCAGGA   CACATTTCCA  
       51   CTATAAATTT   ACAAAGTTAA   ATTTAAAGC   TAGCATTAAG   TAAAGTGAAG  
      101   TTCCAGCTCC   CTTGTCTAAA   ATAACATAGG   GTAATAATTC   GTATTTCAGGT  
      151   AACTCATTTA   CATCATATAATG   TGTGTGAAA   A

## TAU12/1(2)

20           1   TATAAAATAT   AAATATATT   ATAAAATCATG   TATTATTTAT   AAATATATAT  
       51   TATAAAATTA   TAAAATATA   AAATTATATT   TAGGCTTAAT   GTATAAAGGA  
      101   TATAAAATTT   TAATAAGCAT   ATGA

## TAU 12/1(1)

25           1   TGTAAATTAAAC   TGTNCNTGTA   GGTCTGTCTT   TTATACATGT   GTGAGTTTTT  
       51   CTTTCACATA   GATTCCTAGC   ATTGGGATTTG   CTAGGTCAAG   TGGTATGCC  
      101   ATTTGACATT   TITGATTGATA   GCACCAGATT   GCTTTGTAA   AAAATTTTNN  
      151   TTTATAGTTT   ACATTATCTT   TGTACAATAG   ATGTTCTCTT   TCGAC

## 30           TAU 12/2(1)

35           1   GGGAAGTGAA   TTGAAAATAC   TTCTTTNTCA   ACATAATTTT   NGGGTTTTGA  
       51   AATTGTTTT   GGGTTTTCAG   GAAATTGOTG   GTAATCTTGT   ATTAGACTGAA  
      101   AAAAGGTGAA   TTTTAAAATT   CTCAGTGAG   AAGCAAAATGA   TTTATTTTC  
      151   ATAGA

## TAU12/3(2)

40           1   TGTCTGGTA   ACTGTCTAA   TTGTGTCTTT   GTTACTTCCA   GTGCAACCT  
       51   TTCAGGTAAG

## TAU12/3(1)

45           1   CTAAAGAACT   TGGTATCTCT   ATTAAGCAC   ACGAACCTCC   AAGGAAATA  
       51   GAGCGATTTA   CTCTTCTCAT   ATCAGTGCAT   ATTTATAAGA   AGCACGGAGT  
      101   CA

## TAU13/1(1)

50           1   AGTCATCAAT   TCCTTTTTAT   CTGTAATTAC   ACATTGTTT   TTATTTCAA  
       51   GTAAATATAA   GGTGTATAT   TCCATATAT   CAGAAAACTA   AATGAAATA  
      101   AAATTTAGT   AAGCCCCGCC   CCTTTGACCG   ATACAGAAAA   CTTGA

## TAU 13/3(2)

5	1	TATATGGCAG	TCTAAAGCAT	CAAAAGATTG	CATCAACATC	TTTCATTTTA
	51	GACATCTCCT	TGCAATGAA	AAATATCATGT	ATCAACAAACA	TCTGGTGCAA
	101	ATCCATGAGT	CTAACCTCGAC	ATTCATCTTA	GCTCGATTAT	TATTCCTTCG
	151	TACAGTCGAT	GTAACAACTA	CAGAAAGAGG	ATTATTAAGA	ACAGITTT
	TAU 13/3(1)					
10	1	ATTCAATGAAA	TGGTCTATAT	GCATGATATT	GTAAATTCCGG	ACTCGAAACC
	51	GAACCAACGG	ATTCGGTAC	AAAAAATTCCT	TAATGCTGAG	AAATGTTCTCA
	101	CCGAAACRAC	ATCATGGACA	TTAAATTCMA	GATATGTGAA	TGTTAATTCCT
	151	GTCATTAAG	TCACCTAA	GAGTAAAGTT	AAAAACAGTT	ATATCTNNNC
15	201	TGTCATATGAT	GAGTTTAGTT	TAACAGATGA	TGAATCAATT	CT

## TCO 16/1(C)

20	1	CAAAGTGT	TTGGTTTTGA	GAGAGAGAGA	GATTGAGAGA	CAGAGAGAGA
	51	GAGAGAACCC	AAAGGATCAT	GATAGTTATA	GTCAAAATACG	AGGTTGGATT
	101	ATCTTTGAA	ATGTTGTGG	TCTGTGATA	CAAGAGGAAG	CTAAGACATA
	151	TGGTGAACAA	ATCTCCCCCC	TCCACCTTAA	TATCAAGAAC	AAATTGTGGA
	201	ATCTAATGTT	AAAGAGAAAGT	AGTTCCCCAC	TGTGTCAGAT	G

25	TCO16/2(C)					
	1	NCATCTGACA	CAGTGGGGAA	CTACTTCTCA	TTAACATTAG	ATTCCACAAAT
	51	TTNNNNTTGA	TATTAAGGN	NNNNNGGAG	ATCGTTTCAC	GATATCGTCT
	101	TAGCTTCTC	TTGTATCACA	GAACCCACAC	ATTCAAAAG	ATAATCCCTC
30	151	CTCCTTTGAA	CTATAACTAT	CATGATCCCT	TGGTTCTCTC	TCTCTCTCTG
	201	CTCTCTCATC	TCTCTCTCTC	TNAAAACCAA		

## TCO17(C)

35	1	ACAGTAGTTA	GGAGTTTCTT	TACTTACAAA	ATCACTGGAA	ATGATTAAT
	51	TGCTTTTCCC	CCTCCCCAGA	GGTGATTTT	TCTTATTTCC	ATATAGTAAA
	101	GTGAGCTTT	TACAGTCGAT	AAATGTGACAT	TTGGAATGCT	TATCAACTGC
	151	ATGTAACAT	TTAATAACCT			

40	TCO18(C)					
	1	GTAATATGTA	TTANNNNGCTG	AAAGAAAAAA	ATTTTCAG	ACCTCTGTTT
	51	TTAAACTGAA	CTTTATCATT	GGCATTTGTTG	GCTTGTGAGT	TGCTGGGATA
45	101	ATTAATATA	ATTAATAAAA	AGACTGAATT	TAATTGCAA	AAAAAAA
	151	AAACAATAGT	GTGGTGAT			

## TCU2/1(1)

50	1	AAAGAAATAT	CCAGTTATT	ACAAGGCCAC	TGATATTTA	AAACGTCCAAA
	51	AGTTTGTATA	AATGGGCTGT	TACCGCTGAG	AATGATGAGG	ATGAGAATGA
	101	TGGTTGAAGG	TTACATTTA	GGAAATGAAG	AAACTTAGAA	AAATTAATATA
	151	AAGACAGTGA	TTAATACAAA	GAAGATT		

## TCU2/2(1)

5	1	CGGGTTAATA	TTATCCTCTA	GTATAAGTGA	ATTACTAGTT	TCTCTTTATT
	51	TAGACAAACA	CACACACACC	AGATAATATA	AACTTAATAA	ATTATCTGTT
	101	AATCTAGATT	TTATTTAAAA	AACTATATT	GAACATTGGT	CTTTCTTGGG
	151	C				

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## TCU9/1(2)

	1	ACATAACAGC	TTTATACAA	TGATAAGGAC	ATATCAATTG	TTTACAAAGA
	51	AAAGTCTAAA	TTTCAGAAC	ATTCAAAGAG	CTAACACAGT	AAAGGTCATG
15	101	CAAGTTCTAG	AATAGTGAAT	CATGACAGAA	CTCATTCAATT	TTATCCTTTA
	151	TCTCC				

## TCU9/2(2)

20	1	AAAGTATGGGT	AGCTAAATT	GCATTAAATT	AAAGTACAT	ATATTCGAC
	51	ACCAACTCTAC	ATCTGTATAC	CTACGGAACT	ATGTTACTA	CACACCCCTTA
	101	AAATGTTTTT	CAAAGCTTA	ATATATTAGA	ACATGTTTTC	ATTTTTCTAT
	151	GGGATGTTAA	TACTATCTA	TGATTAAGAA	AATACTAG	

25

## TCU10(2)

	1	AATAACAGTA	TCTCTAGCTT	TCATATTCAA	TTGGAATGAT	CAGAAAGATA
	51	TATTAAGTCAC	ACAGAAATTAA	ATATTTAGA	TAGTAAGAAT	C

30

## TCU14(2)

	1	GAAGTGAAAG	TCAGCCCCTT	AGCTTATT	TATTGCTTTA	TTAGAGCAGA
	51	GGGAAGTGCAC	ACTCATGCC	TTCACAGAGC	TCTGCAGAAA	TATATGCCAA
	101	GAGTGCGCAA	TGCCAACATC	TGACTAAGTC	TTCCAAA	

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## TGO20(2)

	1	CAGAACATTA	GGATTTATTC	CTTGATTAGT	TCAAATGATT	TCAACAGCTG
	51	AATTCCCTGA	GATGTGTAA	GCAGCTTGGT	CCTTTGGATG	GACTGTAGAC
	101	TGAAACTTCC	TATAACTGT	CTGATATGTA	CACAGCTACA	TAGCAAAGTC
40	151	CTTCATTATG	AAAATGAAGA	A		

45

## TGO20(1)

	1	CAGTGTGAGA	CTCTCATTC	TATGCCACAGT	CTTTCCTCAGG	AGGATGGAGC
	51	TAGTTAGCTG	TCTGTTGCT	GTAGCCCAGC	TTGATAATGG	AACTATACAG
	101	CGAAGAGACA	ATCTCTGGCA	AGTTTTGTGA	GAA	

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## TGUS5(C)

	1	TTAGACTAAA	ATTCCAAATA	ATGCTTTGC	TCCAAAATTA	CACTAACCG
	51	GCTGGCTTC	TATCATACAT	CTTCATAC	CTCAAAACCTA	GATTGTAAG
	101	TGAAAAAAGT	GATTACNNNT	TCCATTGTT	CATTCTGTCA	CTCACATTCT
	151	TAGGCATTT	AAGGATGAGC	AACTTTGTT	TCAGAAAGGG	TAAGTAATT
	201	GCCCCCTGGA	GGTTACATAG	TTATAATTTA	CTCTTCAGAA	TCCGTTCCGAA

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251 GGGNNNNNGTT ACTATTTTA AGATAATTAG AACCCACCTT GTAGCAATAA  
 301 AAGTTTTCTT GTCTTTG

5

TGU8(2)

1 GCGAAGAAGACT AATCGAACCA TCTAGTAGCT GGTTCCCTCC GAAGTTCCC  
 51 TCAGGATA

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TGU9/1(2)

1 TTAATGTTTA AATACTACTT TTTTTCAAG CTTGCCCTAG ATACCAACTG  
 51 TTTATCTAAC ACACAATTCC AGTGTGCCA AGCCTCATGC CAATTGAG  
 101 GGAAACGCCA AAACTTATGC ATTCAATAAA AAAGAGTCTC TAGGCTCTA  
 151 TATCTACATT ATAATTTT

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TGU9/2(2)

1 GGAAATAACAT TTTTTTATGA GGGAAACCTT TAAAATGGAT GCACACAGTG  
 20 51 GCATTTTCTC CTAGGCTAA AGCTGAGTAC ACTCCCGTAA TTAAATATAAT  
 101 ATTTTAGGCA AGTCCTATGA CAATTATAAC AACAAAGTTTC TTCAACCCCA  
 151 CCACCAACCC ACCATCTCTA TGC

25

TGU12(C)

1 GGAGGAAGCT TTATTTGGGA AGAGTGGCGT TCNTCGGCC CTGATCAGCT  
 51 CTAGCCTGCC CACCCCACTC CACCCAGGCC GCTTTACTTC TTCTGAGCT  
 101 TCAGGTTCTT CTCTCTCTG ATTCCCTGG CGAGCTCCCC AATCAATCTC  
 151 CAGTACTCAT TGAAACTTGAG CTCCGAGNCC TGATTACAT CCAAGCTCTT  
 30 201 CATCTTCT

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TGU13/1(C)

1 GGATGTTGTA GTTGATCTT AATGCCATT CTAGGTCGGA AAAATCCATG  
 51 ATCCTAACTT TAAGAGAAG GTGGTAACT CTACTTAGGA CTTTTTTTG  
 101 TAAGAGGAAT AATGTTAGCT CACCCCTATC TTTCCTGGAA TGTTTAAACC  
 151 ACTGAAATAT GGAGATCAA TCCAGCTTAC ACAGTGGTAA CTCAAATACT  
 201 ATTTTTTTT TAAACTTAATC TTTCCTAAACT AATCACCCCT CTGTTACATA  
 40 251 GAACCTTCTA TCTCAGTGCCTT AATTCCTAGA GGTTGATGCA AACAGCTCTC  
 301 CAGAGAGCCT GTGCTATTGT TC

TGU13/2(2)

1 GGGGTGTACA TTTTATTGGA AACCTTAAT AACTGTTCAGA AAGAATATAT  
 45 51 CTTCACATCAA GGTCCTGCCG AGCCCTACACA GAAAAAATGAA GCTTTTTGGG  
 101 TTAGGGGCMA GGATATATAC AGTACAGAGG ACAAAAGA

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TTO16/2(C)

1 ACATTCACTA AAGATGAAGT TTCAAGCATCT TCACATTGAG ATCCCATCAGA  
 51 TGATTCCTGAG AGGCAAGGTCT CCCCCAAAAA TCCACCCGAT GTATTCCTTC  
 101 GTTTAGAATC TGAAGGCCTC TTCTTTCTA GGCTTGATGA CTCTTCTAAG  
 151 GTATTTGTTA TGCCCTCTCTT CTGGGTTTTT CGTTTGCCT TATCAAGTAG

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	201	CTNAATTCA	AACACCATGG	CAANAGAAC	TGCTTCTAT	
5	TTO20/1(C)					
	1	CCACCAAGCTT	ACTGATCAGC	TGGGATGCTC	CTGCTGTCAC	AGTGGAGATAT
	51	TACAGGATCA	CTTACGGAGA	AACAGGAGGA	ATAAGCCCTG	TCCAGGAGTT
10	101	CACTGTGCC	GGGAGCAGT	CTACAGCTAC	CATCAGGGCC	CTTAAACCTG
	151	GAGTTGATTA	TACCATCACT	GTGATGCTG	TCACGGCCG	TGGAGACAGC
15						
	201	CCCGCAAGCA	GCAAGCCAA	TTCCATTAA	TACCGAACAG	AAATTGACAA
	251	ACCATCCCAG	ATGCAAGTGA	CCGATGTTCA	AGACAACTGT	TTTAATAAAA
	301	GATTTACATT	CCAC			
20	TTO20/2(2)					
	1	TTGGTACCC	AGTCACAGAA	CTGGGGTCA	TTTCTAGAT	GAACAAACG
	51	GAACAGTT	TCTTCCAAACA	AAGAAATGTA	CTGTAGAAAT	TAATTTCTC
25	101	CATGAAATT	ATATATTGTC	TACAAATATA	AGGTATGTTAT	CTGAAATACAA
	151	AG				
	TTU2/1(2)					
25	1	CTAGAACTTC	CAAAGGCTGC	TTGTCATAGA	AGCCATTGCA	TCTATAAAGC
	51	AAACGGCTCTT	GTAAATGGT	ATCTCCTTC	TGAGGCTCC	ACTAAAAGTC
	101	ATTTGTTACC	AAACCTTAT	GTGCTTAA	AGGCCAATG	TTCTCG
30	TTU 2/2(C)					
	1	AAACGTTATT	TCAAAACTAT	TATCTGGATT	CAAGATTAGT	GTGTAAGAT
	51	TGTTTTCTTA	TCAGTAAAT	AGGCTTCAG	ATCTGCATCT	GGCCTCTAG
	101	CATGTTTT	TTCATAGATA	CCCGTTTGG	GTTTTTGCG	TCGGAAGATG
	151	AAAGTGCAGT	TATAGTCCTC	TCCACATTA	TCTG	
35	TTU3(1)					
	1	GGGTAGAAAAG	CTGAATAATT	TATGAAGGAG	AGGGGTCA	GTTGATTCCG
	51	AGGGACCTAT	TGGTCGGGG	GCTTTGTATG	ATTATGGGCG	TTGATTAGTA
40	101	GTACTTACTG	GTGAACATT	GTTTGTGGT	GTATATATTG	TAATTGAGAT
	151	TGCTCGGGGG	AAATGGTTAT	GTGATTAGGA	GTAGGGTTAG	GATGAGTGGG
	201	AAG				
	TTU 5/1(2)					
45	1	GACAAAAAAA	AAAAAACAGG	TTTTAAAGCT	AGAAATGAA	AGCTACTTAA
	51	GTATCTTAA	GGATAAGTTA	CTTATTATA	CACTGAAAC	ATACACACATA
	101	GCTGAAACT	TAACAAATCT	CACACTGCTG	AATGTCCTCG	CTGGCTG
50	TTU5/2(2)					
	1	GCATCCATTG	TACATTGTTT	GGTTTGAGGT	TACCATGAGG	CCTGTAATA
	51	CTATCTTATA	ATTTATTATT	TCAACCTGAT	AAAACCTAAC	ACTATTGCA
	101	AAACACAAACA	AACGAAAAA			

## TTU9/1(1)

5           1   TAAAAATACTG   GTTCTTTAT   TCTGCAATAT   TTTAAAAAT   CACATTTCA  
      51   GCCAGGCAGCA   GTTTCCCACA   CCTGTAATCC   GGCACTTGG   GAGGCTGAGA  
      101   TGGGTGGATC   ACAAGGTAGG   AGATCAAACA   TCCTGGCCAA   CATGGTGAAC  
      151   CTGTTTACT

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## TTU9/2(2)

15           1   CAAGTATGGG   TAGCTAAATT   TGCATTAAT   TAAAAGTACA   TATAATGCAA  
      51   CACCACCTCA   CATCTGTATA   CCTACGAATG   TATGTGTAATC   ACACACCCCT  
      101   AAATGTTCA   AAGCTTAATA   TATAGAACAA   TGTTTTCATC   TTCAGGGAG

## TTU13(2)

20           1   GGAAATACAC   TAGCATGTGA   GCACGTGATA   TAAAGCTTGA   GGTTAGGAGG  
      51   TAAATGAAA   GAAATCATT   TTAACTCCTA   AGATGT

## TTU13(1)

25           1   TGAATTAAT   GGACTCGTTG   AAAGGACAAG   GAGATCGGTA   ATATCTCTCT  
      51   AAAGAACTTA   TATACTAAA   TCTGTAATTG   CCTGTACCAA   AAGTTTTAGT  
      101   CTTCCTTT

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or an analog thereof. In accordance with the invention, the term "analog" includes nucleic acids which code for the same protein sequence due to the degeneracy of the genetic code, for a protein having conservative amino acids substitutions or deletions that do not eliminate the characteristic feature of this protein, or for a protein having at least about 85 %, and more advantageously at least about 90 %, in particular 95 % amino acid sequence homology.

Other embodiments of the invention provide a vector containing said DNA and a host cell containing said vector.

According to the general knowledge one skilled in the art can also use said nucleic acids of the present invention as a hybridization probe to detect the corresponding genes in an organism or in a sample from an organism or gene mutations thereof.

Therefore, an additional embodiment is a method for isolating a gene which can be induced or repressed by treating chondrocytes that contain this gene by IL-1 $\beta$  containing the steps:

45           (a) hybridizing a DNA of the present invention under stringent, preferably high stringent conditions against DNA or RNA containing said gene, preferably DNA or RNA isolated originally from chondrocytes, in particular human chondrocytes; and

50           (b) isolating this gene by methods known to a skilled person in the art.

According to the present invention the term "stringent conditions" means hybridization conditions comprising a salt concentration of 4 x SSC (NaCl-citrate buffer) at 62-66°C, and "high stringent conditions" means hybridization conditions comprising a salt concentration of 0,1 x SSC at 68°C. The length of the probes are 6-100, preferably 10-40, in particular 12-25 nucleic acids long.

Yet another embodiment is a process for expressing a gene isolated according to the above-described process containing the steps:

55           (a) cloning said gene into a suitable expression vector such as the pET series (Studier et al., 1990. Methods in Enzymology 185, 60) for procaryotic expression or the vector CDM8 for mammalian expression (Aruffo and Seed, 1987. Proc. Natl. Acad. Sci. USA 84, 8573) or any other expression system known to one skilled in the art; and

(b) expressing said gene in a suitable host cell such as BL21 series (Studier et al., 1990, *supra*) for prokaryotic expression or COS cells for mammalian expression (Aruffo and Seed, 1987, *supra*) or any other expression system known to one skilled in the art;

5 or a method for producing a protein containing the steps:

(a) culturing a suitable host cell, in particular the above mentioned, containing a vector, in particular an expression vector such as the vectors mentioned above which contains a DNA or a gene of the present invention; and

10 (b) isolating the expressed protein for example by ultrafiltration, precipitation with chaotropic agents such as urea or column chromatography on e.g. ion exchange chromatography columns as detailed in Ausubel et al. 1994 (*supra*).

A further embodiment is a diagnostic aid containing a DNA or parts thereof or a gene or parts thereof of the present invention. In particular, quantification of the genes can be achieved on the RNA level by Northern blotting with gene specific probes of the present invention or with gene specific primers in a PCR reaction. Such primers can be synthetically produced using the DNA sequences of the present invention or the sequences of the corresponding genes. Therefore, said nucleic acids are useful for the diagnosis of IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

These nucleic acids can also be used to evaluate the expression of certain genes in small cartilage biopsies and 20 to use these ultimately as disease-specific markers and/or as predictive markers for disease progression of e.g. osteoarthritis. The hybridization conditions can be the same as described above.

Said nucleic acids, however, can also be used for the therapy against the diseases mentioned or for the production 25 of a pharmaceutical.

Therefore, another embodiment of the present invention is also the use of said nucleic acids for the production of a pharmaceutical. For example, as described by Uhlmann & Peyman (Chem. Rev. (1990), 90, 543), Milligan et al. (J. Med. Chem. (1993), 36, 1923) or Stein & Cheng (Science (1993), 261, 1004) such nucleic acids can be used as antisense oligonucleotides or triple helix forming oligonucleotides for the inhibition of gene expression. This is in particular useful if such a disease is caused by the overproduction of a gene product which is directly or indirectly regulated by IL-1 $\beta$  in chondrocytes. The nucleic acids can additionally be modified in order to increase e.g. the stability against nucleases as 30 described e.g. in the literatures mentioned above.

Finally, also the gene product itself produced by a method of the present invention can be used as a pharmaceutical.

In the following the invention is in particular described by the examples and tables.

#### Description of the Tables

35 Table 1 gives an overview on used primers and the complexity of the detected differences in expression.

Table 2 summarizes the result of the sequencing of differentially displayed PCR products after their elution from the sequencing gel, reamplification and subcloning into the pCRII vector. The sequences of TAU1/1(1) and TAU1/1(2) are 100 % identical to human osteopontin cDNA, the sequence of TTU2/2 is 97.2 % identical to human calnexin. bp = base 40 pairs, IL-1 = Interleukin-1 stimulation, Stat. sig. score = statistical significance score: a feature of the BLAST database searching program. This score is determined using an implementation of Karlin's significance formula (Karlin, S. and Altschul, S.F. 1990. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc. Natl. Acad. Sci. USA, 87:2264-2268), which calculates the Poisson probability that the observed sequence similarity will occur by chance based on the size and composition of the sequence database as well as on the size and quality of the match. The smaller this number, the more it is likely to see sequence similarities.

#### Examples

#### Cell culture

50 Articular cartilage specimen were obtained from two patients (male 65 years old and female 73 years old) undergoing total joint replacement surgery for osteoarthritis. None of these individuals had received treatment by radiation or chemotherapy. Articular cartilage slices were aseptically dissected from both femoral condyles, tibia plateaus and patellae and subjected to sequential enzymatic digestion with pronase and collagenase as described (Häuselmann HJ et al. 1992, Matrix 12, 116-129). Since it is known that the alginate gel suspension system retains the chondrogenic phenotype [Lohmander LS et al. 1992, Trans. Orthop. Res. Soc. 17, 273]  $4 \times 10^6$  chondrocytes were suspended in low viscosity alginate ( $4 \times 10^6$  cells / ml 1.25 % w/v alginate in an isotonic buffered solution) and expressed through a 22gauche needle into 102 mM CaCl solution to form cell entrapping beads which are 1.5-3 mm in diameter and spherical. Alginate beads containing a total number of  $2 \times 10^7$  cells were fed daily for the first three days with medium F12 / DMEM (50/50)

and 10 % fetal calf serum (Sigma) with 25 µg / ml ascorbate and 50 µg / ml gentamycin and were then subdivided into two populations for further three culture days in the presence or absence of 5U / ml rh IL-1 $\beta$  (Genzyme). For cell recovery, alginate beads were finally dissolved into dissolution buffer (55 mM sodiumcitrate, 30 mM EDTA, 0,15 M NACl) and placed at room temperature for 10 min. Viability was checked by eosin-red exclusion and cell number was determined.

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#### Primer syntheses

Arbitrary oligodecamer primers OPA6 to OPA10, OPA16 to OPA20 and degenerate anchored oligo-dT primers (T<sub>12</sub>VN) were synthesized using the 392 DNA synthesizer (Applied Biosystems) and purified by denaturing polyacrylamid gel electrophoresis. Some oligodecamer primers, U1 to U15 were purchased from Biometra (Göttingen, FRG).

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5 List of all degenerate 3' oligo dT-primers [T<sub>12</sub>VN] used for DDRT-PCR:

Primer	Sequence 5' to 3'
T <sub>12</sub> VA	5'-TTTTTTTTTTTTVA-3'
T <sub>12</sub> VA	5'-TTTTTTTTTTTTVT-3'
T <sub>12</sub> VA	5'-TATTTTTTTTTTVG-3'
T <sub>12</sub> VA	5'-TTTTTTTTTTTTVC-3'
V = dA, dG, dC; N = dA, dT, dG, dC	

10 List of all arbitrary 5' oligodecamer primers used for DDRT-PCR:

Primer	Sequence 5' to 3'
OPA 6	GGTCCCCCTGAC
OPA 7	GAAACGGGTG
OPA 8	GTGACGGGTG
OPA 9	GCGTAAACGCC
OPA 10	GTGATCGCAG
OPA 16	AGCCAGCGAA
OPA 17	GACCGCTTGT
OPA 18	AGGTGACCGT
OPA 19	CAACACGTGG
OPA 20	GTTGCGATCC
U1	TACAACGAGG
U2	TGGATTGGTC
U3	CTTTCTACCC
U4	TTTTGGCTCC
U5	GGAACCAATC
U6	AAACTCCGTC
U7	TCGATACAGG
U8	TGGTAAAGGG
U9	TCGGTCATAG
U10	GGTACTAAGG
U11	TACCTAACGCG
U12	CTGCTTGATG
U13	GTTTTCGCAG
U14	GATCAAGTCC
U15	GATCCAGTAC

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## RNA isolation and cDNA synthesis

5 Total RNA from cultured articular chondrocytes was prepared according to a single step method Chomczynski and Sacchi (Chomczynski P & Sacchi N 1987, *Anal. Biochem.* 162, 156-159) and incubated with 10 U RNasefree DNaseI (Gibco, Eggenstein, FRG) for 30 min at 37°C to remove chromosomal DNA contamination of RNA. After extraction with phenol/chloroform (3:1), the supernatant was ethanol precipitated in the presence of 0.3 M NaOAc and RNA was redissolved in DEPC treated water. 0.4 µg total RNA was then reverse transcribed using 200 U M-MLV (Moloney murine leukemia virus) reverse transcriptase (Gibco, Eggenstein, FRG) in a 40 µl reaction volume containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM DTT, 3 mM MgCl<sub>2</sub>, dNTP mix (dATP, dTTP, dCTP, dGTP) of 200 µM each, 40 U RNase inhibitor (Boehringer Mannheim, FRG) and 2.5 µM degenerate oligo-dT primer (T<sub>12</sub>VN) at 37°C for 1 h. Reactions were terminated by heating for 5 min at 95°C.

## PCR amplification

15 cDNAs were amplified in a DNA thermal cycler (Perkin Elmer, model 480) in 20 µl PCR reactions containing 2.5 µM of the original T<sub>12</sub>MN-primer used in cDNA synthesis in combination with 0.5 µM arbitrary upstream primer, dNTP mix (dGTP, dCTP, dTTP) of 0.5 µM each, 10 µCi  $\alpha$ -[<sup>32</sup>P]dATP (1000 Ci/mmol, 10 Ci/ml), 10 mM Tris-HCl (pH 8.3) 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.001 % gelatin and 2.5 U AmpliTaq DNA polymerase. Light mineral oil was overlaid and thermal cycling was performed as follows: 94°C for 30 seconds, 40°C for 2 min and 72°C for 30 seconds for 40 cycles followed by 5 min postextension at 72°C. AmpliTaq DNA polymerase was purchased from Perkin-Elmer (Weiterstadt, FRG) and  $\alpha$ -[<sup>32</sup>P]dATP was obtained from Amersham-Bucher (Braunschweig, FRG). After addition of 5 µl stop buffer (95 % formamide, 20 mM EDTA, 0.05 % bromphenolblue and 0.05 % xylene cyanol) radiolabeled PCR-fragments were then displayed on 6 % acrylamide/7 M Urea high resolution sequencing gels of 35 x 43 cm in size; dried gels were exposed to X-ray film (Kodak X-OMAT) and exposed for 48 h, which allows rapid identification of differentially expressed genes 25 by side by side comparison of DDT-PCR band patterns.

## Elution, reamplification and cloning of PCR fragments

30 PCR fragments identified as differentially expressed bands were cut from acrylamide gels, transferred into Eppendorf tubes and rehydrated for 10 min with 100 µl 10 mM Tris-HCl and 1 mM EDTA at room temperature. After boiling the gel slice for 15 min, the PCR fragment was recovered by ethanol precipitation in the presence of 0.3 M NaAc and 20 µg glycogen as a carrier and redissolved in 10 µl sterile water. 5 µl of this volume was used for reamplification by PCR using appropriate primers and conditions described above except for dNTP concentration of 20 µM and no radioisotope. The reamplified PCR product was visualized by electrophoresis on a 2 % agarose gel and eluted from the gel by ultrafiltration 35 using Ultrafree MC-filters (Millipore). Purified PCR fragments were then cloned into the pCRII-vector (Invitrogen, De Schelp, NL) by the TA cloning method (Kovalic D et al. 1991, *Nucleic Acids Research* 19, 4640), which allows in-vitro transcription and sequencing from the plasmid.

## Sequencing

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Plasmid DNA sequencing of subcloned PCR fragments with either SP6(2) or T7(1) primer was carried out using the chain-termination DNA sequencing method, as described by Sanger et al. (Sanger F et al. 1977, *Proc. Natl. Acad. Sci. USA* 74, 5463-5467).

## 45 Sequence analysis

The sequence analysis revealed the sequences of cDNA clones TAO8/2(2), TAO16/1(2), TAO16/2(2), TAO17(c), TAO19(c), TAU1/1(2), TAU1/1(1), TAU1/2(2), TAU7/1(2), TAU7/1(1), TAU7/2(c), TAU10(1), TAU12/1(2), TAU12/1(1), TAU12/2(1), TAU12/3(2), TAU12/3(1), TAU13/1(1), TAU13/3(2), TAU13/3(1), TCO16(6), TCO16/2(c), TCO17(c), TCO18(c), TCU2/1(1), TCU2/2(1), TCU9/1(2), TCU9/2(2), TCU10/2(2), TCU14(1), TCU14(2), TGO20(2), TGO20(1), TGU5(c), TGU8(2), TGU8/1(2), TGU9/2(2), TGU12(c), TGU13/1(c), TGU13/2(2), TTO16/2(c), TTO20/1(c), TTO20/2(2), TTU2/1(2), TTU2/2(c), TTU3(1), TTU5/1(2), TTU5/2(2), TTU9/1(1), TTU9/2(2), TTU13(2), TTU13(1) disclosed on pages 7 to 14 of the specification.

50 Searching for homology between subcloned PCR fragments and sequences already listed in one of the DNA databases (GenBank or EMBL database) was performed using the FASTA program developed by Pearson and Lipman (Pearson W & Lipman DJ 1988, *Proc. Natl. Acad. Sci. USA* 85, 2444-2448) included in the GCG software package (Genetics Computer Group, Madison, USA).

## Northern blot analysis

Cell culture and isolation of RNA was performed exactly as described above. 10 µg of total RNA from both IL-1 $\beta$  stimulated or not stimulated chondrocytes were denatured by heating at 65°C for 10 min in a solution of 50 % formamide, 5 20 mM MOPS and 2.2 M formaldehyde, separated through a 1 % agarose gel containing 2.2 M formaldehyde in 1 X MOPS and transferred to positively charged nylon membrane (Amersham) by standard blotting procedures [Maniatis et. al 1992]. After UV crosslinking, the blots were prehybridized for 1 h in rapid-hyb-buffer (Amersham) at 65°C. A 330 bp cDNA corresponding to nts 61 to 390 of human osteopontin cDNA (GenBank J04765) and a 340 bp cDNA corresponding to nts 881 to 1220 from human calnexin (GenBank M94859) were radiolabeled for hybridization with  $\alpha$ -[32P]dCTP (3000 Ci/mmol, 10 mCi/ml) using random nonamer primers (Amersham) up to a specific activity of  $\sim 1.5 \times 10^9$  dpm /  $\mu$ g DNA. Hybridization was performed for 2.5 h at 65°C in prehybridization solution with 2 ng / ml of labeled probe added. The blot was subsequently washed in 2 X SSC, 0.1 % SDS at 37°C for 15 min (1 X SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0), followed by two successive washes with 1 X SSC, 0.1 % SDS at 65°C for 10 min respectively. If necessary, a final high stringency wash was performed with 0.1 X SSC, 0.1 % SDS at 65°C for 15 min. The blots were then analysed 15 by autoradiography using Kodak X-Omat films at -80°C with intensifying screens for 2-7 days and intensity of bands was quantified with a phosphorimager (Biorad, model GS-250). All blots were stripped with boiling 0.5 % SDS solution and reprobed with labeled  $\beta$ -actin to demonstrate equal loading of RNA in each lane.

## Northern hybridisations (Results)

20 Fragment TAU7/2(c), identical to TSG-6, was differentially upregulated in IL-1 stimulated cells. This is in concordance with Lee et al. (1992) which reported for TSG-6 a TNF- $\alpha$  and IL-1 mediated upregulation. Fragment TAU1/1, identical with human osteopontin and fragment TAU2/2, identical to human calnexin, both were weaker expressed in IL-1 stimulated chondrocytes compared with the unstimulated cells. To validate our differential display data, we performed Northern 25 analyses of Osteopontin and calnexin expression in IL-1 stimulated and unstimulated chondrocytes originating from a third patient. Both messages were again downregulated. A phosphorimager quantification revealed an osteopontin downregulation by 79% and a calnexin downregulation by 40% in the RNA population from chondrocytes of the third

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Table 1: Current results of differential display/ reverse transcriptase PCR (DDRT-PCR) to reveal differential gene expression by chondrocytes with and without IL-1 $\beta$

Overview on used primers and number of analysed bands			reproducibility of DDRT-PCR band pattern from first to second, third or reamplified in PCR (as in patient <sup>1</sup> ) (other patient <sup>2</sup> )	eluted from gel and cloned into PCR vector by TA cloning method verified by PCR sequencing using SP6	[PCR fragment sequenced using PCR or T7 promoter]
DDRT-PCR primercombination	3'-Oligo dT-primer (downstream primer)	5'-Oligodecamer (upstream primer)			
T <sub>12</sub> M <sup>*</sup> A	OPA 6 - OPA 10	25 out of ~ 4000 bands	7	6	1
T <sub>12</sub> M <sup>*</sup> T	OPA 16 - OPA 20	19 out of ~ 4000 bands	13	9	12
T <sub>12</sub> M <sup>*</sup> G	U 1 - U 5	31 out of ~ 4000 bands not done	11	11	10
T <sub>12</sub> M <sup>*</sup> C	U 6 - U 10	27 out of ~ 4000 bands not done	13	12	11
	U 11 - U 15	21 out of ~ 4000 bands not done	11	11	10
total 4 x	25	total 123	55	total 52	total 44
= 100 combinations					

\* means threefold degeneracy where M may be dA, dG or dC

<sup>1</sup> patient female 73 years old diagnosis genuarthrosis<sup>2</sup> patient male between 65-75 years old

theoretical consideration:

Suggesting that an arbitrary upstream primer detects 3 % of the total RNAs (Lang 1994), then 37 % of the total mRNAs will not be detected, i.e. with 25 arbitrary oligodecamerprimer and the four degenerate T<sub>12</sub>M<sup>\*</sup>N primers, about half of the mRNAs would be seen ( $P = 1 - (0.97)^{25} = 1 - 0.977^{25} = 53.3\% \text{ in 100 lanes of high resolution sequencing gels}$ .

Table 2 IL-1 mediated differentially displayed cDNA fragments of human articular chondrocytes

Fragment	bp	IL-1	Features	Stat.sig.score
TAO 8/2(2)	275 bp	+	146 bp sequenced, no homology found	0.999
TAO 16/1(2)	450 bp	+	80 bp sequenced, no homology found	0.69
TAO 16/2(2)	200 bp	+	115 bp sequenced, no homology found	0.04
TAO 17(c)	412 bp	+	412 bp sequenced, no homology found	0.016
TAO 19(c)	209 bp	--	209 bp sequenced, no homology found	0.99
TAU 1/1(1,2)	450 bp	--	100 % sequence identity to human osteopontin cDNA in 303 bp overlap (303 bp seq.)	$1.2 \times 10^{-101}$
TAU 1/2(2)	430 bp	+	188 bp sequenced, no homology found	0.82
TAU 7/1(1,2)	500 bp	+	87 % sequence identity to human cDNA clone c-1sd02 in 125 bp overlap (235 bp seq.)	$8.1 \times 10^{-33}$
TAU7/2(c)	202 bp	+	99.5 % sequence id to human TNF stimulated gene-6 in 202 bp overlap	$4.8 \times 10^{-78}$
TAU 10(1)	400 bp	+	181 bp sequenced, no homology found	0.9997
TAU 12/1(1,2)	470 bp	--	319 bp sequenced, no homology found	$3.3 \times 10^{-14}$
TAU 12/2(1)	390 bp	--	155 bp sequenced, no homology found	0.0078
TAU 12/3(1,2)	250 bp	--	95 % sequence identity to human cDNA clone HRBBA21 similar to S10 in 158 bp overlap (162 bp seq.)	$1.0 \times 10^{-28}$
TAU 13/1(1)	600 bp	+	145 bp sequenced, no homology found	0.12
TAU 13/3(1,2)	500 bp	--	438 bp sequenced, no homology found	0.33
TCO 16/1(c)	241 bp	+	241 bp sequenced, no homology found	$2.4 \times 10^{-7}$
TCO 16/2(c)	230 bp	+	230 bp sequenced, no homology found	$4.3 \times 10^{-6}$
TCO 17(c)	169 bp	+	169 bp sequenced, no homology found	0.49
TCO 18(c)	168 bp	+	168 bp sequenced, no homology found	$1.3 \times 10^{-6}$
TCU 2/1(1)	400 bp	+	178 bp sequenced, no homology found	0.66
TCU 2/2(1)	210 bp	+	151 bp sequenced, no homology found	0.0074
TCU 9/1(2)	430 bp	+	99 % sequence identity to human cDNA clone 131036 3' in 155 bp overlap (155 bp seq.)	$7.2 \times 10^{-58}$
TCU 9/2(2)	320 bp	--	188 bp sequenced, no homology found	0.22
TCU 10(2)	320 bp	--	100 % sequence identity to human cDNA clone 26518 3' in 85 bp overlap (91 bp seq.)	$2.9 \times 10^{-28}$

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	Fragment	bp	IL-1	Features	Stat.sig.score
5	TCU 14(1,2)	280 bp	+	99.3 % sequence identity to human cDNA HL60 3' directed MboI in 249 bp overlap (249 bp seq.)	$3.5 \times 10^{-51}$
10	TGU 20(1,2)	300 bp	+	304 bp sequenced, no homology found	0.95
	TGU 5(c)	317 bp	+	317 bp sequenced, no homology found	0.088
	TGU 8(2)	320 bp	+	100 % sequence identity to human 28S rRNA in 58 bp overlap (58 bp seq.)	$1.4 \times 10^{-16}$
15	TGU 9/1(2)	280 bp	+	169 bp sequenced, no homology found	0.55
	TGU 9/2(2)	220 bp	--	100 % sequence identity to human cDNA clone 12A10B in 100 bp overlap (173 bp seq.)	$4.0 \times 10^{-36}$
20	TGU 12(c)	208 bp	--	87 % sequence identity to human cDNA clone 113442 3' in 208 bp overlap	$5.5 \times 10^{-43}$
	TGU 13/1(c)	322 bp	+	322 bp sequenced, no homology found	$6.9 \times 10^{-13}$
25	TGU 13/2(2)	300 bp	--	94.9 % sequence identity to human F1 ATPase $\beta$ -subunit in 137 bp overlap (137 bp seq.)	$2.3 \times 10^{-43}$
30	TTO 16/2(c)	239 bp	+	97.5 % sequence identity to human ERCC5 in 239 bp overlap (239 bp seq.)	$9.3 \times 10^{-88}$
	TTO 20/1(c)	314 bp	+	100 % sequence identity to human fibronectin in 314bp overlap (314 bp seq.)	$1.9 \times 10^{-121}$
35	TTO 20/2(2)	400 bp	+	152 bp sequenced, no homology found	0.035
	TTU 2/1(2)	300 bp	--	100 % sequence identity to human cDNA clone 118470 5' in 146 bp overlap (146 bp seq.)	$2.1 \times 10^{-36}$
40	TTU 2/2(c)	184 bp	--	99 % sequence identity to human calnexin in 184 bp overlap (184 bp seq.)	$2.3 \times 10^{-64}$
45	TTU3(I)	400 bp	+	97 % sequence identity to human NADH-DH mtDNA subunit in 203 bp overlap (203 bp seq.)	$8.6 \times 10^{-99}$
50	TTU 5/1(2)	300 bp	--	147 bp sequenced, no homology found	0.0065
	TTU 5/2(2)	270 bp	--	118 bp sequenced, no homology found	0.035

Fragment	bp	IL-1	Features	Stat.sig.score
TTU 9/1(1)	350 bp	+	94 % sequence identity to human cDNA clone 83764 3' in 159 bp overlap (159 bp seq.)	5,9 x 10 <sup>-23</sup>
TTU 9/2(2)	320 bp	--	149 bp sequenced, no homology found	0,22
TTU 13(1,2)	350 bp	+	194 bp sequenced, no homology found	0,57

Thus, the 44 identified fragments can be subdivided as follows:

1) 2 fragments with sequence homologies to known human genes with known roles in IL-1 mediated processes:

TAU 7/2 identical with human TNF-stimulated gene-6  
 TTO 20/1 identical with human fibronectin

2) 6 fragments with sequence homologies to known human genes, whose function in IL-1 mediated processes can be speculated:

TAU 1/1 identical with human osteopontin  
 TGU 8 identical with human 28S ribosomal RNA gene  
 TGU 13/2 identical with human F1 ATPase  $\beta$ -subunit  
 TTO 16/2 identical with human ERCC5  
 TTU 2/2 identical with human calnexin  
 TTU 3 identical with human NADH-DH mtDNA subunit

3) 9 fragments with sequence homologies to human genes, identified in human genome sequencing projects:

TAU 7/1 identical with human cDNA clone c-1sd02  
 TAU 12/3 identical with human cDNA clone HRBBA21  
 TCU 9/1 identical with human cDNA clone 131036 3'  
 TCU 10 identical with human cDNA clone 26518 3'  
 TCU 14 identical with human cDNA clone H160 3' directed MboI  
 TGU 9/2 identical with human cDNA clone 12A10B  
 TGU 12 identical with human cDNA clone 113442 3'  
 TTU 2/1 identical with human cDNA clone 118470 5'  
 TTU 9/1 identical with human cDNA clone 83764 3'

4) 27 fragments without sequence homologies to known human genes. The detection of TSG-6 and fibronectin, both genes known to be upregulated by IL-1, points to the importance of those other cDNA fragments in the light of IL-1 mediated processes. Those genes very likely play roles in degenerative joint diseases, including rheumatoid and osteoarthritis and with this are interesting candidates as markers for clinical studies or as drug targets for pharmacological intervention.

#### Claims

1. Use of osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  a mediated diseases of connective tissues, in particular osteoarthritis.
2. Diagnostic aid for the diagnosis of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.

3. Pharmaceutical for the prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.

5 4. Use of calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis.

10 5. Diagnostic aid for the diagnosis of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.

15 6. Pharmaceutical for the prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.

20 7. Use of TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis.

25 8. Diagnostic aid for the diagnosis of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.

9. Pharmaceutical for the prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.

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10. DNA containing a DNA selected from the group consisting of

TA08/2(2)

5                   1 CCAAGTTTT CCAGCAACCC CAAGGGATAA CAGGGAGATC AATGCACCCA  
       51 AAATGGAAA AGAAAAATAC TTTCGATGCAA TGAAAACAAG CCTTTTCCG  
       101 TTTCAGTTCC ATAATTCAGT GGTCAGTTT AAGGCTGCCA CTTGGG

10               TA016/1(2)

1               1 GACACGAACA CCACATATT TTATTGGAGG CCCCCATGGCT CCTTGGAAAGC  
       51 CATTGGAA CCAAGGGAC CCACCTTTT

15               TA016/2(2)

1               1 CTAATAATAT TCTCTAACAA GTTAATCTCT TTCAAATCTA TAGATAAAAC  
       51 TAAAGGATA AGGAAACCAAG GTTTAACCGA CCTAGCCAAT TATGGCAATC  
       101 ATACTTGCTT TTTAG

20               TA017(C)

1               1 CATGAAATAT TTCTTGAGGT ATAAGCTTT TACCAAGCTT ATATTTTG  
       51 GCAATTCACT TACATGAGA AAAAAACACA CCAAAAGACCC AAAAATTATA  
       101 AAAACTCACT TTCTTGCAA TCATAGACAT TTGCATTATT ATAGAACATT  
       151 CAAACAAAGTT AGGTGGATAA TTATTTGCTA TAGATAAAATA CGATGCAATT  
       201 TTAATAAGAA TTGARAGAT GACATTAAT GCTGTCGAA GCCTTTGAT  
       251 TTTTAATGT ATGACCGATA CTCCGTATAT ACTTAGATAA CTTATCCAGA  
       301 AACCTCAACT GTATTGAACA TTGCTGAGAG AAATCAACAA TAATTTAAC

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351 AGATATGATG ACAGNAAAAA TTGATTGCAT ATCTTTTGC ACTAAAACCTT  
 401 TTATATTTAT TT

5 TAO19(C)

1 AGAGCAGGGG TATTCNCGG TTCAATACCGC CATGGCTTAA GAAGCAAAAG  
 51 TCATATACCT TAGTAGTGGC AAGATNGAG GAGATAAAA AGAGCCTACC  
 101 CAAGCTGTT TTGAGAACAA GGCTCTTGGAT AAGAGGAAC CCTTCCAGAA  
 151 GNACAGAGAC AGGCTAAGGG TGATGCTGAG GAAATGGCTC AGAAGAAACA  
 201 AGAGATTAA

15 TAU 1/1(2)

1 CTAAATGCAA AGTGAGAAAT TGTATTTTTT CTCCCTTTAA TTGACCTCAG  
 51 AAGATGCACT ATCTAATTCA TGAGAAATAC GAAATTTCA GGTGTTATCT  
 101 TCTCCCTAC TTTTGGGG

20 TAU 1/1(1)

1 ACATCACCTC ACACATGGAA AGCGAGGGAGT TGAATGGTGC ATACAAGGCC  
 51 ATCCCGGTTT CCCAGGACCT GAACCGCCT TCTGATTGGG ACACCGCTGG  
 101 GAAGGACAGT TATGAAACGA GTCAAGCTGGA TGACCAAGAGT GCTGAAACCC  
 151 ACAGGCCAACAA GCAGTCCGAA TTATATAAGC GAAA

TAU1/2(2)

1 CGGGAATGGG GAGCAAACATA TAAGAACCGG GACCAGTTTC CTCTCTTTGT  
 51 GCTCTAGGTC CCCCTCTTT GTATCACACCC TCCATCTGGA ATAGACTCTG  
 101 GTTCTCAGGG TAACACGGAC AACATCAAT CCTGTAGAGA AACAAATGTT  
 151 AGCTCAGAAC GACACAGCCTC TTGAATCATC AGAGAGTT

35 TAU 7/1(2)

1 GTTAAAGAATA ACTAAATAAA AGTTTTAATT TATTAGGAA TATAAAAAAC  
 51 TATTAACATT TAATTTATA ACTGTATCTG CCAAGCAACT TTAATATATA  
 101 TTTTATTTAC

40 TAU 7/1(1)

1 CACGCAATGT GAAATAGGCA CATAGGAAGA ATGGGAAAC CATCCCTCA  
 51 AGCATTTATC CTITGAGGTA CAAGCAATCC AATTACACTC TTTAGTTAT  
 101 TTTTAAATGT ACAGTTAGGT TATTA

45 TAU 7/2(C)

1 CCTGGAAGAT GACCCAGGT NCTTGGCTGA TTATGTTGAA ATATATGACA  
 51 GTTACGATGA TGTCCATGGC TTGTTGGGAA GATACTGTGG AGATGAGCTT  
 101 CCAGATGACA TCATCAGTAC AGGAATGTC ATGACCTTGA AGTTCTAAG  
 151 TGATGCTTCA GTGACAGCTG GAGGTTCCA AATCAAAATAT GTGCAATGG  
 201 AT

5 TAU10(1)

1 GGAGATGACA TTTGCTTTGG GCAGAGGCAG CTAGCCAGGA CACATTTCCA  
 51 CTATAAATTT ACAAAAGTTAA ATTTATAAGC TAGCATTAAG TAAAGTGAAG  
 101 TTCCAGCTCC CTTGCTAAAA ATAACATAGAG GTAATAATTG GTATTCCAGGT  
 151 AACTCATTTA CATCATAATG TGTGTGAAA A

10 TAU12/1(2)

1 TATAAAATAT AAATTATATT ATAATCATG TATTATTTAT AAATTATAT  
 51 TATAAAATTAA TAAAAATATA AATTATATT TAGGCTTAAT GTATAAGGAA  
 101 TATAAAATTAT TAATAAGCAT ATGA

15 TAU 12/1(1)

1 TGTAATTAAC TGTNCTTGTA GGTCTGCTT TTATACATGT GTGAGTTTT  
 51 CTTTACAATA GATTCCTAGC ATGGGGATG CTAGGTCAAGA TGGTATGCCAC  
 20 101 ATTTGACATT TTGATTGATA GCACCAAGATT GCTTTGTTAA AAAATTTNN  
 151 TTATAGTTT ACATTATCTT TGTACAATAG ATGTTCTCTT TCGAC

25 TAU 12/2(1)

1 GGGAAAGTGA TTGAAAATAC TTCTTTNTCA ACATAAATTT NGGGTTTTGA  
 51 AATTGTGTT GGGTTTTAG GAAATGGGTG GTAATCTTGT ATTAGCTGAA  
 101 AAAAAGTGA TTTAAAATT CTCACTGAAG AAGCAATGA TTTATTTTC  
 151 ATAGA

30 TAU12/3(2)

1 TGTTCTGGTA ACTGTTCTAA TTGTGTCTTT GTTACTTCCA GTGCAACCCCT  
 51 TTCAGGTAAAG

35 TAU12/3(1)

1 CTAAGAACT TGGTATCTCT ATTAAGCAC ACGAACCTCC AAGGAAAATA  
 51 GAGCGATTAA CTCTTCTCAT ATCAGTGCAT ATTTATAAGA AGCACGGAGT  
 40 101 CA

TAU13/1(1)

1 AGTCATCAAT TCTTTTTAT CTGTAATTAC ACATTTGTTT TTATTTCAA  
 45 51 GTAAATTATAA GGTGTTATAT TGCATATAAT CAGAAAACATA AATGGAAAATA  
 101 AAATTTAGT AAGCCCCGCC CCTTGTACCG ATACAGAAAAA CTTGA

TAU 13/3(2)

1 TATATGGCAG TCTAAAGCAT CAAAGATTG CATCAACATC TTTCATTTTA  
 50 51 GACATCTCT TGCATGTAA AATATCATGT ATCAACACAA TCTGGTCAA  
 101 ATCCATGAGT CTAACCTGCAT ATTCACTTAA GCTCGATTAT TATTCCTCG  
 151 TACAGTCGAT GTAAACATA CAGAAAGAGG ATTATTAAGA ACAGTTT

TAU 13/3(1)

5           1 ATTCATGAAA TGGTCTATAT GCATGATATT GTAAATTCCG ACTCGAAACC  
 51        51 GAAACCAAGG ATTCCGTTAC AAAAATTCTT TAATGCTGAG AATGTTCTCA  
 101      101 CGAAACACAC ATCATGGACA TTAAATTCAA GATATGGAA TTGTTAATCT  
 151      151 GTCAATAAAG TCAACGTAAG GAGTAAGTT AAAAACAGTT ATATCTNNNC  
 201      201 TGTCAATGAT GAGTTAGTT TAACAGATGA TGAATCAATT CT

10

TCO 16/1(C)

15           1 CAAAGTGT TTGGGTTTGAGAGAGAGA GATGGAGAGA CAGAGAGAGA  
 51        51 GAGGAAACCC AAGGGGATCAT GATAGTTATA GTCAAAATACG AGGTTGGATT  
 101      101 ATCTTTGAA AATGTGTTGG TTCTGIGATA CAAAGGAAAG CTAAGACATA  
 151      151 TCGTGGAAAC ATCTCCCCCC TCCACCTTAA TATCAAGAAC AAAATGTGGA  
 201      201 ATCTAAATGTT AATGAGAACT AGTTCCCCAC TGTGTCAGAT G

20

TCO16/2(C)

25           1 NCATCTGACA CAGTGGGAA CTACTTCTCA TTAACATTAG ATTCACAAAT  
 51        51 TTNNNCTTGA TATTAAGGNN NNNNNNGGAG ATCGTTTCAC GATATCGTCT  
 101      101 TAGCTTCTC TTGTATCACA GAACCAACAC ATTTCAAAAG ATATCCTTC  
 151      151 CTNNNCTTGA CTATAACTAT CATGATCCCT TGGTTCTCTC TCTCTCTCTG  
 201      201 CTCTCTCATC TCTCTCTCTC TNAAAACCAA

30

TCO17(C)

35           1 ACAGTAGTTA GGAGTTCTT TACTTACAAA ATCACTGGAA ATGATTAAT  
 51        51 TCGTTTTCCTT CCTCCCCAGA GGTCGATTTT TCTTATTTCCTT ATATAGTAAA  
 101      101 GTTGAGCTT TACAGTGCAT AATGTGACAT TTGGAATGCT TATCAACTGC  
 151      151 ATGTAACAT TAATAACCT

40

TCO18(C)

35           1 GTAAATGGTA TTANNNNGCTG AGAAAAAAA ATTTTCAAG ACCTCTGTTT  
 51        51 TTTAACGAA CTTTATCATG GGCATCTGTTG GCTTTGAAGT TGCTGGGATA  
 101      101 AATTAAATATA ATAAATAAA AGACTGATT TAACTGCAAA AAAAAAAA  
 151      151 AACAAATAAGT GTGGTGAT

45

TCU2/1(1)

50           1 AAGAAATTAT CCAGTTATT ACAAGGCCAC TGATATTATA AACCTCCAAA  
 51        51 AGTTGTTTA AATGGGCTGT TACCGCTGAG AATGATGAGG ATGAGAATGA  
 101      101 TGGTTGAAGG TTACATTATA GGAAATGAAAG AAACCTTAGAA AATTAATATA  
 151      151 AAGACAGTGA TAAATACAAA GAAGATT

55

TCU2/2(1)

50           1 CGGGTTAATA TTATCCTCTA GTATAAGTGA ATTACTAGTT TCTCTTTATT  
 51        51 TAGACAAACA CACACACCC AGATAATATA AACTTAATAA ATATCTGTT  
 101      101 AATGTGAGATT TTATTTAAA AACTATATTG GAACATTGGT CTTCTTGGA  
 151      151 C

## TCU9/1(2)

5           1 ACATAACAGC TTTTATACAA TGATAAGGAC ATATCATTTG TTTACAAAAGA  
 51          51 AAGCTAAAAA TTTCAGAAC ATTCAAGAG CTAACACAGT AAAGGTCATG  
 101        101 CAAAGTCTAG AATAGTGAAT CATGACAGAA CTCATTCTT TTATCCTTTA  
 151       151 TCTCC

10           TCU9/2(2)  
 1          1 AAGTATGGGT AGCTAAATT GCATTAATT AAAAGTACAT ATAATGCAAC  
 51        51 ACCACTCTAC ATCTGTATAC CTACGAATGT ATGTTGACTA CACACCCCTTA  
 101      101 AATGTTTTT CAAAGCTTAA ATATATAGA ACATGTTTC ATTTTTTCTAT  
 151      151 GGGATGTTAA TACTATTCTA TGATTAAGAA AATACTAG

20           TCU10(2)  
 1          1 AATACAGTTA TTCTAGCTT TCATATTCAA TTGAAATGAT CAGAAAAAGTA  
 51        51 TATTAGTCAC ACAGAATTAA ATATTTAGA TAGTAAAGAT C

25           TCU14(1)  
 1          1 ATCCTTAGTA AGTGGATTTT GGGGAAAAAA GCACCTGGGC TTCTGGTTCT  
 51        51 TTTTGATAAT ATATAAATT ATTCAATTATG AGGTTGCCAGT TGTTTGCAAA

30           TCU14(2)  
 1          1 GAAATGAAAG TCAGCCCTTT AGCTATTATT TATTGCTTTA TTAGAGCAGA  
 51        51 GGGAAATGAC ACTCTATGCC TTCAACAGGC TCTGCAAGAAA TATATGCACA  
 101      101 GAGTGGTCAA TGCCAACATC TGAGTAAAGTC TTCCAAA

35           TGO20(2)  
 1          1 CAGAACATTA GGATTATTTC CTTGATTAGT TCAAATGATT TCAACAGCTG  
 51        51 AATTCTCTGA GATGTGTAAG GCAGGTTGGT CCTTTGGATG GACTGTAGAC  
 101      101 TGAAACTTCC TATAACTGTA GTGATATGTA CACAGCTACA TAGCAAAGTG  
 151      151 CTTCATTATG AATATGAAAGA A

40           TGO20(1)  
 1          1 CAGTGTGAGA GTCTCATTTTC TATGCACAGT GTTTCTCAGG AGGATGGAGC  
 51        51 TAGTTAGCTG TCTGTTGCT GTAGCCCCAGC TTGATAATGG AACTATACAG  
 101      101 CGAAGAGACA ATCTCTGCCA ACTTTTGTA GAA

45           TGUS(C)  
 1          1 TTAGAGTAAT ATTCCAAATA ATGCTTTGC TCCAAAATTA CACTAACAG  
 51        51 GCTGGCTCTC TATCATACAT CTTCAAATACC CTCAAACCTA GATTGTTAAAG  
 101      101 TGAAGAAAGT GATTAGCCTT TCCATTGTTT CATTCTGTCA CTCACATCT  
 151      151 TAGGCATTTT AAGGATGAGC AACCTTTGTT TCAGAAAGGG TAAGTAATTA  
 201      201 GCCCCCTGGA GGTACATAG TTATAATTAA GTCTTCAGAA TCCGTTGCAA  
 251      251 GGGNNNNGTT ACTATTTTA AGATAATTAG AACCCACCTT GTAGCAATAA  
 301      301 AAGTTTCTT GTCTTTG

## TGUB8(2)

5 1 GCGAAAGACT AATCGAACCA TCTAGTAGCT GGTTCCCTCC GAAGTTTCCC  
 51 TCAGGATA

## TGU9/1(2)

10 1 TTAATTTTA AATACTACTT TTTTTCAAG CTTGCCCTAG ATACCAACTG  
 51 TTTATCTAAC ACACAAATTCG AGTGTGGCA AGCCTCATGC CAATTGAG  
 101 GGAACAGCCA AAACITATGC ATTCAATATAA AAAGAGTCTC TAGGCTTTA  
 151 TATCTACATT ATAATTTT

## TGU9/2(2)

15 1 GGAATAACAT TTTTTATGA GGGAAACCTT TAAATGGAT GCACACAGTG  
 51 GCATTTCTC CTAGGCTCAA AGCTGAGTAC ACTCCCGTAA TTTTAATAAT  
 101 ATTTTAGGCA AGTCTATGA CAATTATACC AACAAAGTTTC TTCAACCCCCA  
 20 151 CCACCCCCC ACCATCTCA TGC

## TGU12(C)

25 1 GGAGGAAGCT TTATTTGGGA AGAGTGGCGT TCNNNTGGCC CTGATCAGCT  
 51 CTAGCCTGCC CACCCCCATC CAGCCAGGGG GCTTTACTTC TTCCCTGAGCT  
 101 TCAAGGTTTT CTGCTTCTGG ATTTCTTGG CCAGGCTCCCC ATCAAACTCTC  
 151 CAGTACTCAT TGAACCTGAG CTCCGAGNCC TGATTCACAT CCAAGCTCTT  
 201 CATCTCTT

## TGU13/1(C)

30 1 GGATGTGGTA GTTGATCTT AATGCCATT CTAGTGGGA AAAATCCATG  
 51 ATCCAACTT TTAAGGAGG GTGGTAACT CTACTTGGAGA CTTTTTTTG  
 101 TAAGAGGAAT AATGATGCTT CACCCCTTATC TTTCTGGAAA TGTTAAACCC  
 151 ACTGAATAT GGAGATCAA TCCAGCTTAC ACACTGGTAA CTCAAACTACT  
 201 ATTTTTTTTT TAAACTATCTT TTCTAAACT AATCACCCCT CTGTACATA  
 251 GAACCTTCTA TCTCAGTGGC AATCTTAGA GTTGTGATGCA AACAGCTCTC  
 301 CAGAGAGCCT GTGCTATTGT TC

## TGU13/2(2)

40 1 GGGGTGTACA TTTTATTGGA AACCTTAAT ACTGTTGAGA AAGAATATAT  
 51 CTTCAATCAA GGTCCTGGCG AGCCTACACA GAAAAATGAA GCTTTTTGGG  
 45 101 TTAGGGCAGA GGATATATAC AGTACAGAGG ACAAAGA

## TTO16/2(C)

50 1 ACATTCATTA AAGATGAACT TTCAGCATCT TCACCTGAG ATCCATCAGA  
 51 TGATTCGTGAG AGGCAGGTCT CCCCCAAAAA TCCACGGCAT GTATTCTTTC  
 101 GTTTAGAATC TGAACGCCCTC TTTCCTTCA GGCTTGATGA CTCTCTAAG  
 151 GTATTTGTTA TGCTCTCTT CTGGGTTTT CGTTTGCT TATCAAGTAG  
 201 CTNAATTCA AACACCATGG CAANAGAAC TGCTTCTAT

## TTO20/1(C)

5           1 CCACAGCCT ACTGATCAGC TGGGATGCTC CTGCTGTAC AGTGAGATAT  
       51 TACAGGATCA CTTACGGAGA AACAGGAGGA AATAGCCCTG TCCAGGAGTT  
 10       101 CACTGTGCCT GGGAGCAAGT CTACAGCTAC CATCAGCGGC CTTAAACCTG  
       151 GAGTGTATTA TACCACTACT GTGTATGTCTG TCACTGGCGG TGGAGACAGC  
 15       201 CCCGCAAGCA GCAAGCCTAAT TTCCATTAAT TACCGAACAG AAATTGACAA  
       251 ACCATCCCAAT ATGCAAGTGA CGCGATGTCA AGACAACTGT TTTAATAAAA  
       301 GATTACATT CCAC

## TTO20/2(2)

15           1 TTGGTACAC AGTCACAGAAA CTGGGGGTCA TTTTCTAGAT GAAACAAACCG  
       51 GAAACAGTTC TCTTCCAAACA AAGAAATGTA CTGTTAGAAAT TAATTCCTC  
 20       101 CATGAAATTT ATATATTGTG TACAAATATA AGGTATGTAT CTGAATACAA  
       151 AG

## TTU2/1(2)

25           1 CTAGAACTTC CAAAGGCTGC TTGTATAGA AGCCATTGCA TCTATAAAGC  
       51 AACGGCTCTT GTTAAATGGT ATCTCCTTTC TGAGGCTCCT ACTAAAAGTC  
       101 ATTTGTTACC TAAACCTTAT GTGCCTTAAC AGGCCATGCG TTCTCG

## TTU 2/2(C)

30           1 AACCACTT TCAAAACTAT TATCTGGATT CAAGATTAGT GTGAAAGAT  
       51 TGTTCCTTA TCAGTAARAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG  
 35       101 CATGTTTTTC TTCAAGATA CCCGTTTGG GGTTCCTTGC CGGAAGATG  
       151 AAGTCAGTT TATAGCTCTC TCCACATTAA TCTG

## TTU3(1)

40           1 GGGTAGAAAG CTGAATAATT TATGAAGGAG AGGGGTCAGG GTTGATTCCGG  
       51 GAGGACCTAT TGGTCCGGGG GCTTTGTATG ATTATGGCG TTGATTAGTA  
 45       101 GTATGGTACTG GTTGAACATT GTTTGTGGT GTATATATG TAATTGGAT  
       151 TGCTCGGGGG AATAGGTTAT GTGATTAGGA GTAGGGTTAG GATGAGTGGG  
       201 AAG

## TTU 5/1(2)

45           1 GACAAAAAAA AAAAAACAGG TTTTAAGCT AGAAATGAAA AGCTACTTAA  
       51 GTATCTAAA GGATAAGTTA CTTTATTATA CACTAGAAC ATACACACATA  
 50       101 GCTGAAACT TAAAAAAATCT CACACTGCTG AATGTCTCTG CTGGCTG

## TTU5/2(2)

1       1 GCATCCATTG TACATTGTTT GTTTGAGGT TACCATGAGG CCTGTAATAA  
       51 CTATCTTATA ATTTATTATT TCAACCTGAT AAAACTTAC ACTATTGCA  
 101 TAAACAAACA AACGAAAA

## TTU9/1(1)

5           1   TAAAATACTG   GTTCTTTAT   TCTGCAATAT   TTTTAAAAAT   CACATTTCA  
       51   GCCAGGGCCA   GTTTCCACCA   CCTGTAATCC   GGCAGTTGG   GAGGCTGAGA  
      101   TGGGTGGATC   ACAAGGTAGG   AGATCAAACA   TCCTGGCCAA   CATGGTGAAC  
      151   CTGTTTACT

## TTU9/2(2)

10           1   CRAGTATGGG   TAGCTAAATT   TGCATTAAT   TAAAGTACA   TATAATGCAA  
       51   CACCACTCTA   CATCTGTATA   CCTACGAATG   TATGTGTACT   ACACACCCCTT  
      101   AAATTTCA   AAGCTTAATA   TATTAGAACAA   TGTTTCATT   TTCAGGGAG

## TTU13(2)

15           1   GGAAATACAC   TAGCATGTGA   GCACTGTATA   TAAAGCTTGA   GGTTAGGAGG  
       51   TAAAATGAAA   GAAATCATT   TTAACCTCCTA   AGATGT

## TTU13(1)

20           1   TGAATTAAAT   GGACTCGTTG   AAAGGACAAG   GAGATCGGTA   ATATCTCTCT  
       51   AAAGAACTTA   TATACTAAAA   TCTGTAATTG   CCTGTACCAA   AAGTTTTAGT  
      101   CTTCTTTT

or an analog thereof.

30 11. Vector containing a DNA according to claim 10.

12. Host cell containing a vector according to claim 11.

35 13. Method for isolating a gene inducible by treating chondrocytes with IL-1 $\beta$  containing the steps:

(a) hybridizing a DNA according to claim 10 under stringent conditions against DNA or RNA containing said gene; and  
      (b) isolating said gene.

40 14. A method according to claim 13 wherein said DNA or RNA has been isolated from chondrocytes, particularly human chondrocytes, that were treated with IL-1 $\beta$ .

15. Process for expressing a gene isolated according to claims 13 or 14 containing the steps:

(a) cloning said gene into a suitable expression vector; and  
      (b) expressing said gene in a suitable host cell.

16. Method for producing a protein containing the steps:

(a) culturing a suitable host cell containing a vector which contains a DNA according to claim 10 or a gene produced by a method according to claim 13 or 14; and  
      (b) isolating the expressed protein.

55 17. Diagnostic aid containing a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof.

18. Use of a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof for the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.
- 5 19. Use of a gene isolated according to claim 13 to 14 for the production of a pharmaceutical.

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